

## Systemic Acquired Resistance in Potato

D.J. Navarre, P. Thomas and C. Brown  
USDA-ARS  
24106 N. Bunn Rd.  
Prosser, WA 99350, U.S.A.

P. Kachroo  
Department of Plant Pathology  
University of Kentucky  
Lexington, KY, U.S.A.

**Keywords:** Salicylic acid, crop protection, pathogen, plant defense, disease resistance

### Abstract

Systemic acquired resistance (SAR) is a defense mechanism that is increasingly being exploited for crop protection. However, successful utilization of SAR will likely require optimization of defense induction for each crop followed by a determination of the pathogens against which SAR is effective. We are examining the capacity of different potato tissues to mount an SAR response. Both free and bound basal salicylic acid (SA) concentrations were measured in leaves, flowers, stems, roots and tubers. SA levels were the highest in leaves and flowers, with concentrations of up to 15 µg/gram fresh weight. Relative to *Arabidopsis* or tobacco, high SA levels were also found in stems, roots and tubers. SAR induction by different SAR elicitors, including harpin and BTH was examined. PR-1 was expressed constitutively, in the absence of elicitation. Little or no increase in PR-1 gene expression was seen after treatment with SAR inducers.

### INTRODUCTION

Systemic Acquired Resistance (SAR) is a process whereby a plant that successfully resists a pathogen becomes highly resistant to subsequent infection not only by the original pathogen, but a wide variety of pathogens; this protection lasts for weeks to months (Dempsey et al., 1999). Salicylic acid (SA) is a key regulator of SAR. Salicylic acid is also involved in R-gene mediated resistance and components of SAR are involved in other disease resistance mechanisms. SA signaling also interacts with jasmonate and ethylene signaling pathways, activating both identical and different sets of genes and having complicated cross-talk (Schenk et al., 2000).

Efforts are now being made to use SAR for crop protection, and over a dozen companies are marketing putative elicitors of SAR. SAR strategies in the field have had mixed results, effective in some crops against certain pathogens, ineffective in other crops or against other pathogens. Effective use of SAR is likely to require optimization for each crop, however the vast majority of SAR research has focused on *Arabidopsis* or tobacco. Furthermore, most SAR research has focused on resistance in leaves, thus much less is known about SAR in non-leaf tissues, particularly in below ground parts of the plant. Tobacco and *Arabidopsis* have basal SA levels below 50 ng/gram fresh weight, whereas plants such as rice and potato (Coquoz et al., 1995) have basal SA levels over a 100-fold higher. In the model SAR systems, SA levels are usually low, increasing to high levels only after the appropriate biotic or abiotic stimulus. The consequences of high basal SA concentrations in terms of the effect on SA signaling are not clear. One study suggested cultivars with higher SA levels have higher field disease resistance (Coquoz et al., 1995), while a different study concluded high basal SA is not a constitutive defense mechanism (Yu et al., 1997). As an initial step towards trying to exploit SAR for disease control in potato we are examining potato SA levels in multiple tissues and exploring plant responsiveness to SA or SA functional analogs.

### MATERIALS AND METHODS

Salicylic acid extraction was based on the method of Gaffney et al. (1993) with modifications to allow for a higher throughput approach and recovery. Anisic acid was used as an internal standard and SA recovery averaged greater than 80%. Results are the average of 3-5 independent extractions. Samples were analyzed on an Agilent 1100 with

DAD and FLD detection, using a Novapak C18 column. Plants were grown in a Conviron CMP4030 growth chamber and fertilized weekly. Potato SAR induction was monitored by Northern blot analysis, using a PR-1 probe from 'Russet Norkotah', labeled by random priming. Total RNA was extracted using Trizol and 10 mg were loaded per lane. BTH was a gift from Syngenta and supplied as a 50% formulation; B-1,3 glucan was a component of VacciPlant that was a gift from Agrimar, and harpin was a component of Messenger, a gift from Eden Bioscience.

## RESULTS AND DISCUSSION

### Salicylic Acid in Potato Leaves

We looked at basal SA levels in the leaves of 'Russet Norkotah', 'Russet Burbank' and 'Umatilla Russet' (Fig. 1). SA concentrations were between 4-6  $\mu\text{g}/\text{gram}$  f.w., which is over 100 fold higher than basal levels in *Arabidopsis* or tobacco. Salicylic acid is usually stored in its glucosylated form in plants with a relatively small percentage in the free form. Of the total leaf SA, over 95% is glucosylated (Fig. 1). However, the free SA concentration in potato leaves is higher than the total basal SA concentration in tobacco or *Arabidopsis*. Age influences SA accumulation with older plants showing higher SA levels (Fig. 2). An approximately 6-fold increase in leaf SA concentrations occurred with age.

### SA Levels in Different Tissues

Because pathogens often target specific tissues we examined SA concentrations in multiple tissues (Fig. 3) of 'Russet Burbank'. The basal SA concentration of a particular tissue may influence defense signaling in that tissue. SA levels were high in all tissues examined although, with the exception of floral tissue, lower than the levels found in leaves. Floral tissue had very high basal SA levels of over 15  $\mu\text{g}/\text{gram}$  fresh weight. The role of SA in root defenses is far less characterized than its role in leaves. Both roots and tubers have high basal SA concentrations and the consequences of this for defense mechanisms against soil-born pathogens remain to be determined. Interestingly, SA is present at high levels in both the flesh and skin of tubers.

### SAR Induction

We compared SAR elicitation in *Arabidopsis* vs. potato. *Arabidopsis* was sprayed with different elicitors of SAR and total RNA extracted 36 hr after treatment (Fig. 4A). BTH treatment causes a large increase in PR-1 transcription, while harpin also gives a significant increase over the control. Potato leaf discs were floated in solutions of the stated compounds and RNA extracted after 24 hr (Fig. 4B). The results are different from those seen in *Arabidopsis* as untreated potato plants have considerable basal expression of PR-1. In some experiments, none of the SAR inducing compounds gave a notable increase over the untreated control, in marked contrast to their action in *Arabidopsis*. This suggests that there are conditions in which potato is less responsive to SAR induction.

## CONCLUSIONS

SA levels and signaling are clearly different in potato from those in *Arabidopsis*. The cultivars examined in this study resemble *Arabidopsis* CPR mutants, with high SA levels and constitutive PR expression. The consequences of this for the potato defense response are not clear. Potato can respond to SAR inducers, although in some instances it exhibited a differential sensitivity to induction of PR genes, suggesting that leaf tissue is not always competent to respond. The factors that govern the competence of potato leaves to respond to PR gene elicitation are unknown. Nor is it clear if plants expressing PR-1 constitutively also have a constitutive SAR in effect and if so, could this basal SAR be activated to an even higher degree in such plants? Future studies will clarify if the high basal SA levels in potato constitute an effective defense or, conversely, if this results in lowered responsiveness to SA and impaired defense signaling.

## ACKNOWLEDGEMENT

This work was partly funded by a grant from the Washington State Potato Commission.

## Literature Cited

- Coquoz, J.L., Buchala, A.J., Meuwly, P. and Mettraux, J.P. 1995. Arachidonic acid induces local but not systemic synthesis of salicylic acid and confers systemic resistance in potato plants to *Phytophthora infestans* and *Alternaria solani*. *Phytopathology* 10:1219-1224.
- Dempsey, D., Shah, J. and Klessig, D.F. 1999. Salicylic acid and disease resistance in plants. *Crit. Rev. Plant Sci.* 18:547-575.
- Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H. and Ryals, J. 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* 261:754-756.
- Schenk P.M., Kazan K, Wilson I., Anderson J.P., Richmond T., Somerville S.C. and Manners J.M. 2000. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. *Proc. Natl. Acad. Sci. USA* 97(21):11655-60.
- Yu, D., Liu, Y., Fan, B., Klessig, D.F. and Chen, Z. 1997. Is the high basal level of salicylic acid important for disease resistance in potato? *Plant Physiol.* 115:343-349.

## Figures

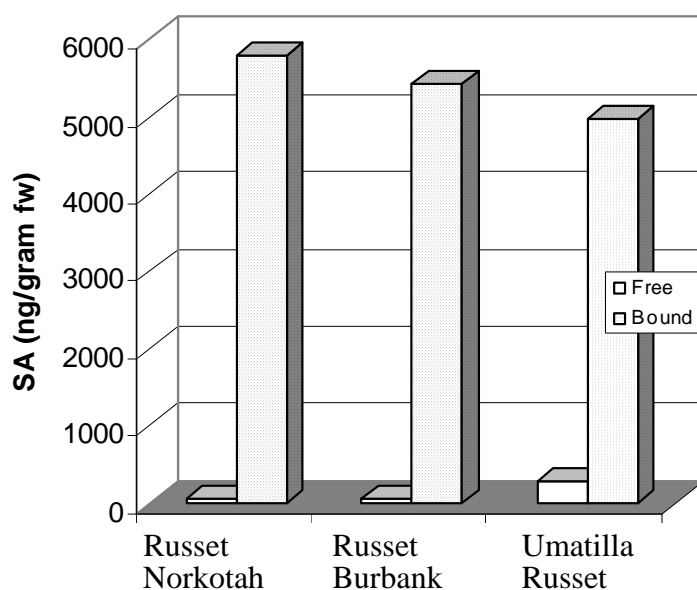


Fig. 1. Free and bound salicylic acid basal concentrations in the leaves of 3 different cultivars.

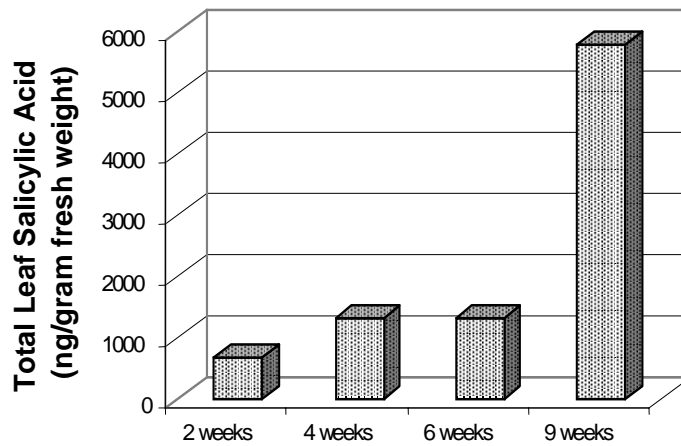


Fig. 2. Total basal SA levels in 2, 4, 6 or 9 week old 'Russet Norkotah'.

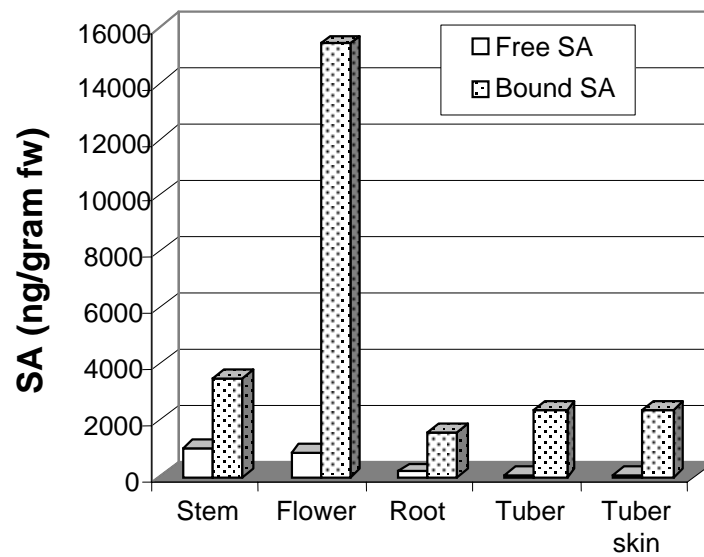


Fig. 3. Free and bound basal SA concentrations in 'Russet Burbank'. SA was extracted from stems, flowers, roots, tubers and tuber skin.

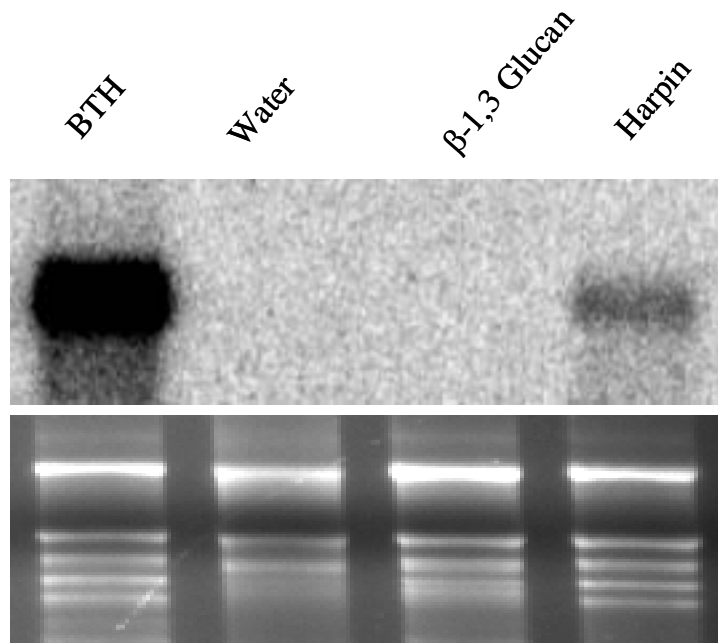


Fig. 4A. Northern blot showing total RNA isolated from *Arabidopsis* 36 hrs after spraying with the indicated treatment of either water, 100  $\mu$ M BTH or 33  $\mu$ g/ml Harpin. B-1,3 glucan was applied as 5.6  $\mu$ l/ml VacciPlant solution. 10  $\mu$ g of total RNA was loaded per lane. The lower panel is the EtBr stained gel and was used as a RNA loading control.

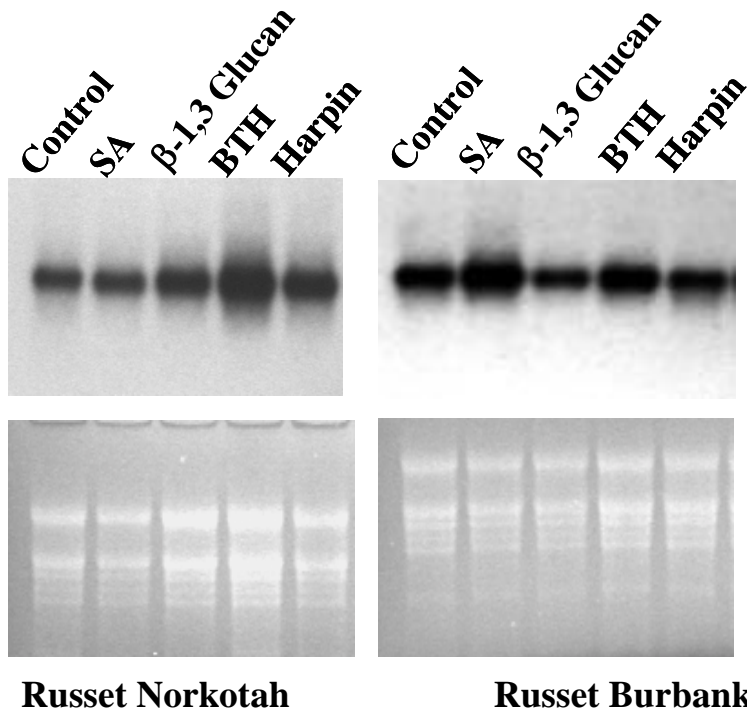


Fig. 4B. PR-1 expression in potato. Leaf discs were floated on the indicated solutions for 24 hr. Concentrations are as described for *Arabidopsis*, except 0.5 mM SA was also used